L Number	Hits	Search Text	DB	Time stamp
1	1	("2003143577").PN.	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2003/08/19 12:20
2	0	("2003143577a1").PN.	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2003/08/19 12:20
3	0	("us2003143577a1").PN.	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2003/08/19 12:21
4	1	(dna adj1 polymerase adj1 compositions).ti.	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2003/08/19 12:28
5	8370	exonuclease and polymerase	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2003/08/19 12:29
6	7502	(exonuclease and polymerase) and (PCR or amplif$8)	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2003/08/19 12:29
7	1379	((exonuclease and polymerase) and (PCR or amplif$8)) and (proofreading (proof adj1 reading))	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2003/08/19 12:29
8	1143	(((exonuclease and polymerase) and (PCR or amplif$8)) and (proofreading (proof adj1 reading))) and fidelity	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2003/08/19 12:29
9	28	((((exonuclease and polymerase) and (PCR or amplif$8)) and (proofreading (proof adj1 reading))) and fidelity) and (lack$4 NEAR proofreading)	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2003/08/19 12:30
10	119	((((exonuclease and polymerase) and (PCR or amplif$8)) and (proofreading (proof adj1 reading))) and fidelity) and (lack$4 NEAR polymerase)	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2003/08/19 12:32
11	210	((((exonuclease and polymerase) and (PCR or amplif$8)) and (proofreading (proof adj1 reading))) and fidelity) and (lack$4 WITH polymerase)	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2003/08/19 12:35
12	62	((((exonuclease and polymerase) and (PCR or amplif$8)) and (proofreading (proof adj1 reading))) and fidelity) and ((lack$4 no) WITH (polymerase NEAR (function activity)))	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2003/08/19 12:35

Detailed Description Text - DETX (172):

The PEF samples tested exhibit no significant DNA polymerase activity while
the Pfu DNA polymerase exhibited a specific activity of 24.times.10.sup.4 u/mg.

Detailed Description Text - DETX (173):

1. Enhancement of Cloned Pfu DNA Polymerase with Pfu PEF

(10) **Patent No.: US 6,183,997 B1**
(45) **Date of Patent: Feb. 6, 2001**

FILE 'BIOSIS, MEDLINE, EMBASE, EMBAL, SCISEARCH, BIOTECHDS, CAPLUS'
ENTERED AT 11:41:03 ON 19 AUG 2003
L1 9698 S **EXONUCLEASE**? AND **POLYMERASE**?
L2 1463 S L1 AND (**PROOFREADING**? OR (PROOF (1W) READING?))
L3 143 S L2 AND (**AMPLIF**? OR **PCR**?)
L4 55 S L3 AND **FIDELITY**
L5 35 DUP REM L4 (20 DUPLICATES REMOVED)
L6 12133 S **EXONUCLEASE**? NOT **POLYMERASE**?
L7 400 S L6 AND (**PCR**? OR **AMPLIF**?)
L8 2 S L7 AND (**PROOFREADING**? OR (PROOF (1W) READING?))
L9 2 DUP REM L8 (0 DUPLICATES REMOVED)
L10 0 S L7 AND (**FIDELITY**)

L5 ANSWER 33 OF 35 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 5
ACCESSION NUMBER: 1992:142409 BIOSIS
DOCUMENT NUMBER: BA93:76634
TITLE: HIGH-**FIDELITY AMPLIF**ICATION USING A THERMOSTABLE DNA **POLYMERASE** ISOLATED FROM PYROCOCCUS-FURIOSUS.
AUTHOR(S): LUNDBERG K S; SHOEMAKER D D; ADAMS M W W; SHORT J M; SORGE J A; MATHUR E J
CORPORATE SOURCE: STRATAGENE INC., 11099 N. TORREY PINES RD., LA JOLLA, CALIF. 92037.
SOURCE: GENE (AMST), (1991) 108 (1), 1-6.
CODEN: GENED6. ISSN: 0378-1119.
FILE SEGMENT: BA; OLD
LANGUAGE: English
TI HIGH-**FIDELITY AMPLIF**ICATION USING A THERMOSTABLE DNA **POLYMERASE** ISOLATED FROM PYROCOCCUS-FURIOSUS.
AB A thermostable DNA **polymerase** which possesses an associated 3'-to-5' **exonuclease** (**proofreading**) activity has been isolated from the hyperthermophilic archaebacterium, Pyrococcus furiosus (Pfu). To test its **fidelity**, we have utilized a genetic assay that directly measures DNA **polymerase fidelity** in vitro during the **polymerase** chain reaction (**PCR**). Our results indicate that **Pcr** performed with the DNA **polymerase** purified from P. furiosus yields **amplif**ication products containing less than 10% of the number of mutations obtained from similar **amplif**ications performed with Taq DNA **polymerase** . The **PCR fidelity** assay is based on the **amplif**ication and cloning of lacI, lacO and lacZ.alpha. gene sequences (lacIOZ.alpha.) using either Pfu or Taq DNA **polymerase**

. Certain mutations within the lacI gene inactivate the Lac repressor protein and permit the expression of .beta.Gal. When plated on. . . per nucleotide induced in the 182 known detectable sites on the lacI gene was 1.6 .times. 10-6 for Pfu DNA **polymerase**, a greater than tenfold improvement over the 2.0 .times. 10-5 error rate for Taq DNA **polymerase**, after approx. 105-fold **amplif**ication.

L5 ANSWER 32 OF 35 MEDLINE on STN
ACCESSION NUMBER: 93113308 MEDLINE
DOCUMENT NUMBER: 93113308 PubMed ID: 1842916
TITLE: DNA **polymerase fidelity** and the **polymerase** chain reaction.
AUTHOR: Eckert K A; Kunkel T A
CORPORATE SOURCE: Laboratory of Molecular Genetics, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.
SOURCE: **PCR** METHODS AND APPLICATIONS, (1991 Aug) 1 (1) 17-24.
Ref: 52
Journal code: 9201445. ISSN: 1054-9803.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199301
ENTRY DATE: Entered STN: 19930219
Last Updated on STN: 19980206
Entered Medline: 19930129
TI DNA **polymerase fidelity** and the **polymerase** chain reaction.
AB High-**fidelity** DNA synthesis conditions are those that exploit the inherent ability of **polymerases** to discriminate against errors. This review has described several experimental approaches for controlling the **fidelity** of enzymatic DNA **amplif**ication . One of the most important parameters to consider is the choice of which **polymerase** to use in **PCR**. As demonstrated by the data in Tables 2 and 3, high-**fidelity** DNA **amplif**ication will be best achieved by using a **polymerase** with an active 3'-->5' **proofreading exonuclease** activity (Fig. 1E). For those enzymes that are **proofreading**-deficient, the in vitro reaction conditions can significantly influence the **polymerase** error rates. To maximize **fidelity** at the dNTP insertion step (Fig. 1A,B), any type of deoxynucleoside triphosphate pool imbalance should be avoided. Similarly, stabilization of errors by

polymerase extension from mispaired or misaligned primer-termini (Fig. 1D) can be minimized by reactions using short synthesis times, low dNTP concentrations, and low enzyme concentrations. Additional improvements in **fidelity** can be made by further manipulating the reaction conditions. To perform high-**fidelity PCR** with Taq **polymerase**, reactions should contain a low MgCl2 concentration, not in large excess over the total concentration of dNTP substrates, and be. . . and pH 7 and a small temperature coefficient (delta pKa/degree C), allowing the pH to be maintained stably throughout the **PCR** cycle. For **amplif**ications in which **fidelity** is the critical issue, one should avoid the concept that conditions generating more DNA product are the better conditions.(ABSTRACT TRUNCATED. . .

L5 ANSWER 13 OF 35 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 2
ACCESSION NUMBER: 2001:378901 BIOSIS
DOCUMENT NUMBER: PREV200100378901
TITLE: Long and accurate **PCR** with a mixture of KOD DNA **polymerase** and its **exonuclease** deficient mutant enzyme.
AUTHOR(S): Nishioka, Motomu; Mizuguchi, Hiroshi; Fujiwara, Shinsuke; Komatsubara, Shusuke; Kitabayashi, Masao; Uemura, Hideki; Takagi, Masahiro; Imanaka, Tadayuki (1)
CORPORATE SOURCE: (1) Department of Synthetic Chemistry and Biological Chemistry, Graduate School of Engineering, Kyoto University, Kyoto, 606-8501: imanaka@sbchem.kyoto-u.ac.jp Japan
SOURCE: Journal of Biotechnology, (15 June, 2001) Vol. 88, No. 2, pp. 141-149. print.
ISSN: 0168-1656.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
TI Long and accurate **PCR** with a mixture of KOD DNA **polymerase** and its **exonuclease** deficient mutant enzyme.
AB DNA **polymerase** from Thermococcus kodakaraensis KOD1 (previously Pyrococcus sp. KOD1) is one of the most efficient thermostable **PCR** enzymes exhibiting higher accuracy and elongation velocity than any other commercially available DNA **polymerase** (M. Takagi et al. (1997) Appl. Environ. Microbiol. 63, 4504-4510). However, when long distance **PCR** (>5 kbp) was performed with KOD DNA **polymerase**, **amplif**ication efficiency (product yield) becomes lower because of its strong 3'-5' **exonuclease** activity for proof-reading. In order to improve a target length limitation in

PCR, mutant DNA **polymerases** with decreased 3'-5' **exonuclease** activity were designed by substituting amino acid residues in conserved **exonuclease** motifs, Exo I (Asp141-Xaa-Glu), Exo II (Asn210-Xaa-Xaa-Xaa-Phe-Asp), and Exo III (Tyr311-Xaa-Xaa-Xaa-Asp). **Exonuclease** activity and **amplification fidelity** (error rate) of the DNA **polymerases** were altered by mutagenesis. However, long and accurate **PCR** by a single-type of mutant DNA **polymerase** was very difficult. The wild-type DNA **polymerase** (WT) and its **exonuclease** deficient mutant (N210D) were mixed in different ratio and their characteristics in **PCR** were examined. When the mixed enzyme (WT and N210D) was made at the ratio of 1:40, long **PCR** (15 kbp) at lower mutation frequency could be efficiently achieved.

L5 ANSWER 1 OF 35 CAPLUS COPYRIGHT 2003 ACS on STN
ACCESSION NUMBER: 2003:571165 CAPLUS
DOCUMENT NUMBER: 139:112720
TITLE: DNA **polymerase** compositions for high-**fidelity** synthesis or **amplification** of DNA
INVENTOR(S): Hogrefe, Holly; Borns, Michael; Sorge, Joseph A.
PATENT ASSIGNEE(S): Stratagene, USA
SOURCE: PCT Int. Appl., 139 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 2
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2003060144	A2	20030724	WO 2002-US40423	20021217
US 2003143577	A1	20030731	US 2002-227110	20020823

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 2001-35091 A 20011221
US 2002-79241 A 20020220

US 2002-208508 A 20020730
US 2002-227110 A 20020823

TI DNA **polymerase** compositions for high-**fidelity** synthesis or **amplification** of DNA

AB The subject invention relates to compns. comprising an enzyme mixt. which comprises a first enzyme and a second enzyme, where the first enzyme comprises a DNA polymn. activity and the second enzyme comprises an 3'.fwdarw.5'-**exonuclease** activity and a reduced DNA polymn. activity. The first enzyme is a DNA **polymerase** with or without 3'.fwdarw.5'-**exonuclease** activity, and preferably Pfu DNA **polymerase** from Pyrococcus furiosus or Taq DNA **polymerase** from Thermus aquaticus, and the second enzyme may be a mutant Pfu DNA **polymerase** with reduced DNA polymn. activity. Mutations are introduced into Pfu DNA **polymerase** that are likely to reduce or eliminate DNA **polymerase** activity, while having minimal effects on **proofreading** activity; the mutations in Pfu DNA **polymerase** are selected from D405E, Y410F, T542P, D543G, K593T, Y595S in the polymn. domain and/or at amino acids 384-389 (SYTGGF) in the partitioning domain. By using a second enzyme with different **proofreading** preference for a nucleotide for the 3'.fwdarw.5'-**exonuclease** activity, one can enhance **proofreading** of the first enzyme by providing **proofreading** to mispairs which the first enzyme is not capable of recognizing and excising efficiently. Thus, for example, the G387P mutation in Pfu **polymerase** yields a relative **exonuclease/polymerase** ratio vs. wild-type of 146.2. Adding Pfu G387G reduced the error rate of DNA synthesis using Taq DNA **polymerase** by 5.1-8.3-fold. The invention also relates to the above compns. in kit format and methods for high **fidelity** DNA synthesis using the subject compns. of the invention.

ST DNA synthesis **amplification** **fidelity** **polymerase** **exonuclease** blend